TRANSPORT OF PALMITIC ACID ACROSS THE GUT OF *ASCARIS LUMBRICOIDES SUUM*

By

GARY ALLEN KING

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1964

Submitted to the Faculty of the Graduate College of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
Master of Science
July, 1966

TRANSPORT OF PALMITIC ACID ACROSS THE GUT OF

ASCARIS LUMBRICOIDES SUUM

Thesis Approved:

Calvin N. Beames, Jr.
Thesis Adviser

Paul J. Cardiellhac

L E McDonald

Dean of the Graduate College

ACKNOWLEDGEMENTS

The author wishes to express appreciation and indebtedness to Dr. Calvin G. Beames, Jr., for his patience, encouragement, and interest throughout this investigation. Without his invaluable assistance this thesis would not have been possible. Gratitude is also acknowledged to the members of the staff of the Department of Physiology and Pharmacology for their interest and guidance. Appreciation is also extended to Dr. Fred A. Hopper, Jr. and Mr. Ben G. Harris for their advice and technical assistance during the course of this study.

A note of appreciation is extended to Mrs. Norella Harris for her assistance in preparing this manuscript.

Finally, the author wishes to express his deepest appreciation to his parents, Mr. and Mrs. Merle A. King, who have been a constant source of encouragement and support throughout this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

There are three important functions of the body lipids of aerobic metazoans (in particular the mammal) (24). These are: (A) a source of potential metabolic energy, (B) protection against excessive loss of heat to the environment, and (C) insulation against mechanical trauma.

Fatty acids are important at the cellular level in two major functions. Fatty acids serve as substrate for oxidative catabolism and production of energy. Due to their high energy equivalence per unit weight, they are important in the storage of nutrient energy. Fatty acids also form complexes with other large molecules such as proteins. These complexes serve as structural components of the cells and of the subcellular organelles. Lipids are especially suitable for such function because of their insolubility in aqueous media (8).

Lipid Transport

The transport of fatty acids, sterols, acyclic fatty alcohols, and hydrocarbons present a problem to the organsim due to their very limited solubility in water (8).

In the past few years knowledge of the mechanisms of transport of lipids across the intestinal mucosa has increased considerably. Information has been obtained concerning the processes that occur in both the

lumen of the intestine and the mucosal cell (13). Hofmann and Borgström (12) have shown that due to the action of pancreatic lipase and the bile salts, lipids are brought in contact with mucosal cells in the form of fatty acids and monoglycerides.

The Gut of Vertebrates and Lipid Transport

All parts of the small intestine of mammals possess villi, which are long finger-like processes of the mucosal that extend into the lumen. The small intestine has villi and crypts of Lieberkühn which are test-tube-like, unbranched, straight glands. The entire surface of the villi and the upper portion of the crypts of Lieberkühn are covered by specialized absorbing epithelium, which is simple striated border columnar epithelium. The epithelial cell surface that is exposed to the lumen of the intestine is formed into numerous finger-like projects called microvilli. Microanatomists refer to the "structure" formed by the microvilli as the brush border of the intestinal mucosa. Goblet cells, which are single mucus-secreting cells are also present in this epithelium (9).

Pancreatic lipolysis generate monoglycerides and fatty acids which interact with bile salt and form mixed bile salt micelles. The brush border of the intestinal mucosal cells then accept the micellar monoglycerides and fatty acids. The bile salt micelles are a means of transportation for the monoglycerides and fatty acids to the brush border from the emulsified lipid phase. The micellar monoglyceride and fatty acid uptake by the intestinal mucosa is a non-energy dependent process (1).

Upon their entry into the cell, the fatty acids and monglycerides

are esterified to triglycerides. This process occurs by two different metabolic pathways. Both pathways are illustrated in Figure 1 (13).

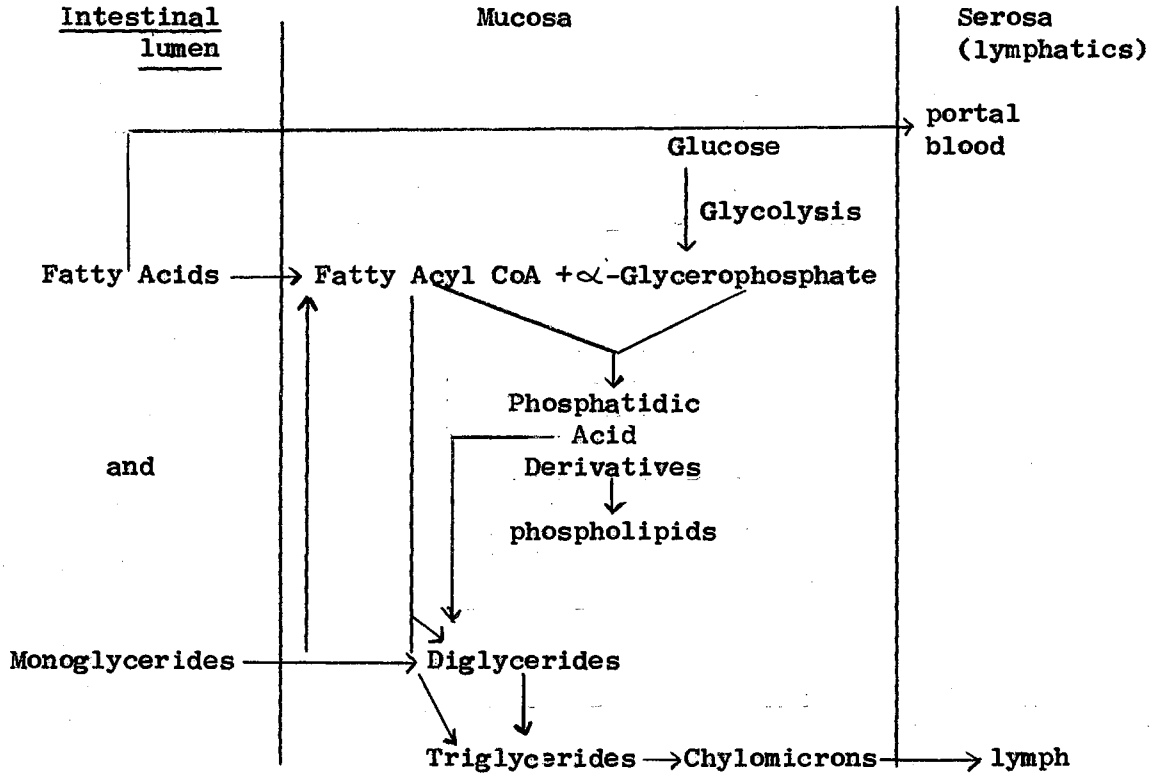


Figure 1. Diagram of the Two Metabolic Pathways Involved in Absorption of Fatty Acids and Monoglycerides Across the Intestinal Mucosa.

The first pathway consists of the acylation of the fatty acids to Acyl-CoA esters. The fatty Acyl-CoA then reacts with α-glycerophosphate to form phosphatidic acid derivatives. The second pathway employes the interaction of monoglycerides with fatty Acyl-CoA molecules to form both diglycerides and triglycerides. The intermediate formation of phospholipids is not involved in the second pathway (13).

Isselbacher (13) determined the biochemical events involved in the conversion of fatty acids to triglycerides in systems of isolated microsomes. The major biochemical reactions he proposes are illustrated in Figure 2.

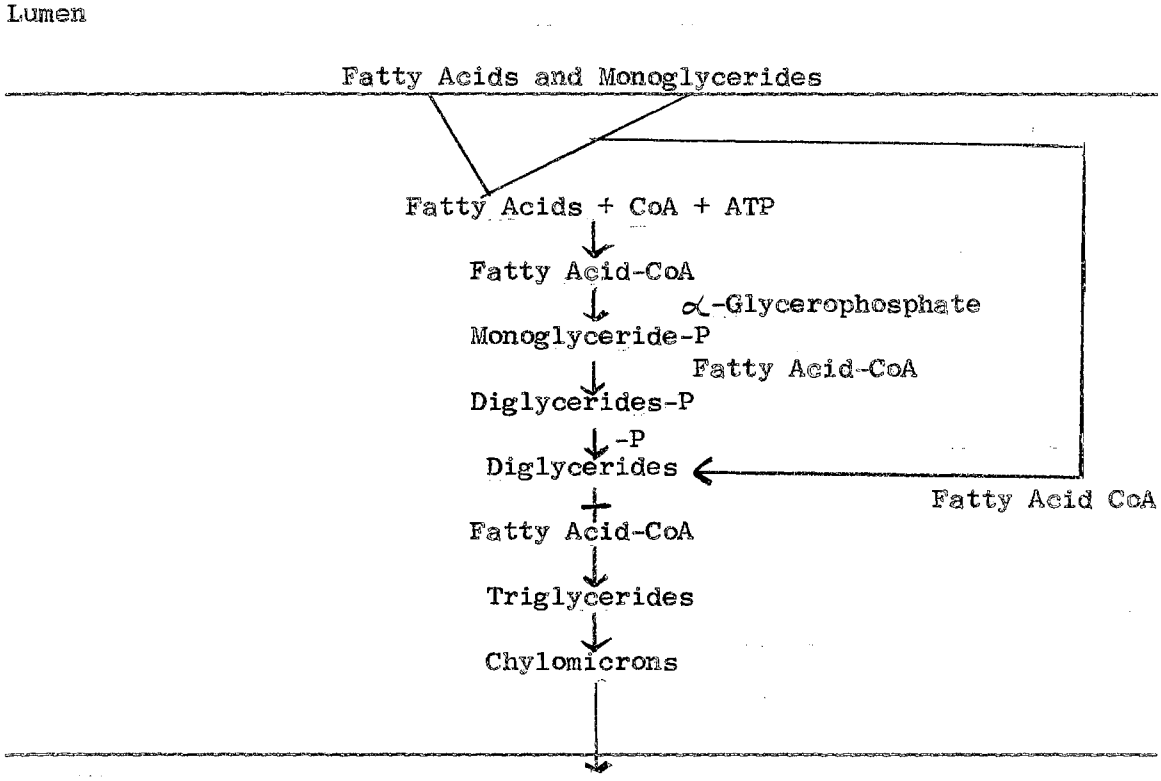


Figure 2. Important Biochemical Reactions Involved in Transport of Fatty Acids and Monoglycerides Across the Intestinal Mucosa.

Isselbacher found that fatty acid esterification involves the interaction of the various fatty acids in the presence of ATP and CoA, to form fatty Acyl-CoA thiolesters (13). The fatty Acyl-CoA thiolesters then react with α-glycerophosphate (synthesized from glucose or glycerol) to form lysophosphatidic acid and finally, phosphatidic acid. Phosphatidic acid is acted on by diglyceride phosphatase to give inorganic phosphate and diglyceride. Free diglyceride is then acylated to form triglyceride (16). The newly formed molecules of triglycerides (0.5 and 1.5 microns in diameter) enter the lymph as minute disbursed droplets termed chylomicrons. A small amount of protein present in the lymph absorbs to the

outer surfaces of the chylomicrons. The presence of the protein increases the chylomicrons suspension stability in the fluid of the lymph and also prevents their adhering to the lymphatic walls. Almost all the cholesterol and phospholipids absorbed from the gastrointestinal tract enter the lymphatic system as chylomicrons, as well as small amounts of phospholipids that are continually synthesized by the intestinal mucosa. Therefore, the chylomicrons which are composed principally of triglycerides also contain small amounts of phospholipids, cholesterol, and protein. As the chylomicrons leave the epithelial cells most of them enter the lymphatic system. A few pass directly to the portal system and are carried directly to the liver. Chylomicrons which are transported up the thoraic duct are emptied into the venous blood at the juncture of the jugular and subclavian veins (10).

Chylomicron lipids are removed from the blood in two ways. The fat is removed by hydrolysis of the chylomicrons triglycerides into glycerol and fatty acids under the influence of an enzyme in the blood called lipoprotein lipase. The glycerol which is metabolized in much the same way as glucose diffuses into the blood, while the fatty acids complex with albumin and are transported to the various cells of the body. Once the fatty acids are taken into the body cells they may be oxidized for energy, or resynthesized into triglycerides. Triglycerides are then stored in adipose tissue to be used later for energy (10). Chylomicrons may be removed from the blood by transport through the capillary wall and directly into the cells. In the cell the chylomicron is hydrolyzed and the fatty acids, etc., may be used either for energy or for forming new structural components (10).

Figure III. Cross-Section of Mid-Gut of Ascaris. Section (10 microns) Stained in Hematoxylin and Counterstained with Eosin.

The Intestine of Ascaris

The alimentary canal of Ascaris is divided into three parts. The portion of the canal which includes the mouth, lips, buccal cavity, and pharynx is termed the stomodaeum. The intestine is divided into the anterior region, the mid-region and the posterior region. Each differs from the other in the shape of the lumen, the height and content of the cells, and possibly the individual function of the cells. A single layer of cells which has a basement membrane on the external surface constitute the wall of the intestine. Numerous microvilli cover the cells internal surface. It is thought that the anterior region of the intestine is secretory and the mid- and posterior regions are absorptive. The rectum and anus of the female and the cloaca and associated structures of the male make up the protodaeum (19).

Carpenter (3) has shown the presence of a lipase like enzyme in the anterior portion of the gut of Ascaris. It was found that glycerol tributyrate was easily hydrolyzed by this tissue and suggested that the anterior gut served a secretory function in Ascaris.

Although little research has been done on transport mechanisms of Ascaris lumbricoides the morphology of the intestine is known in some detail. Intestinal cells of Ascaris have been studied by the use of both the light microscope and electron microscope. Studies with the light microscope, show that the intestinal wall consists of a single layer of epithelial cells which posses a bacillary layer on their internal surface as shown in Figure III. This bacillary layer is composed of numerous filiform extension. In 1950, Chitwood (4) described the presences of a plasma cap which is a thin layer of very

dense cytoplasm that is thickened on the side of the cell that faces the lumen. A very distinct basal lamella and a mesenterial membrane covers the external surface of the cell. The presence of mitochondria which seem to be concentrated in the form of an inverted triangle which lies in the apical end of the cell, a nucleus which is a portion of the terminal bar, and granular inclusions have also been found with the use of the light microscope (17) as shown in Figure III.

The electron microscope has revealed that the bacillary layer is composed of microvilli, which are minute cylindrical projections approximately 6 μ long and 0.1 μ wide (18). The plasma cap is a simple basal extension of the core of the minute microvilli (17). Electronmicrographs have also revealed that the individual intestinal cells are separated from each other by a distinct dense membrane (17). Chitwood (4) proposed that the basal lamella is differentiated as a supporting structure or possibly it is simply a secretion product of the intestinal epithelium. Cytochemical tests have shown that lipid appears to be in greatest concentration in the central portion of the intestinal cell, but lipid has been found to be presence though out the entire cell (17).

The simplicity of the intestine of the Ascaris makes it favorable for transport studies. The intestine of the Ascaris is large enough and strong enough to be removed and used as a sac or compartment preparation. Fisher (personnel communication) has developed a technique which he used to study amino acid and sugar transport in the intestine of Ascaris. He found the system to transport both compounds for a limited period of time. However, all of the experiments were conducted under aerobic conditions. Harpur, (11) has recently reported that oxygen at partial pressures equal

to air at atmospheric pressure inhibit Ascaris tissue. The gut epitheluim is the most sensitive of all tissues studied. It seems quite possible that the time limits observed by Fisher are due to oxygen in the environment. Feist, Read, and Fisher (6) have investigated the synthesis and hydrolysis of trehalose in Ascaris. Their results show that trehalose synthesis does not occur in minces of gut tissue or 50% perienteric fluid (hemolymph) that have been incubated with C^{14} glucose. They also found that trehalose was in a very low concentration while glucose was in a high concentration. Fisher's observations (personnel communication) indicate that glucose is the compound on the antiluminal side of the intestine during glucose absorption. This supports the idea that trehalose synthesis is absent in the intestine of the Ascaris. Feist, et. al (6) did find the gut to be the only tissue in Ascaris where trehalose was hydrolyzed. Since trehalose is hydrolyzed to glucose in the gut, this glucose may be used as a source of energy for the transport of materials across the gut.

At the present time, there is no available information concerning the movement of lipid across the gut of Ascaris. The high rate of egg production by the adult female worm indicates that there is either a high rate of lipid synthesis or that the worm is able to acquire lipids from the host very efficiently. The purpose of this study was to determine the ability of Ascaris gut to transport lipids from the mucosal to the serosal surface. In addition, experiments were carried out to determine some of the factors that may influence the transport of fats across the gut.

CHAPTER II

MATERIALS AND METHODS

Preparation of Fatty Acid-albumin Complex: The fatty acid-albumin complex was prepared according to Johnston (15). 1.0 ml of 0.1 M palmitic acid and 0.4 ml of palmitic acid-1-C^{14}(36.6mc/mM) were dissolved in a small amount of ethyl ether and mixed with 4 ml of 95% ethanol. The solution was neutralized to a pH of 7.0 with 0.1 N NaOH and was taken to near dryness in vacuo at 40°C. Ten ml of a 0.5% solution of albumin in an Ascaris holding saline recommeded by Harpur (11) was added and the mixture was shaken mechanically for 1 hour at 37°C. The solution was filtered through a Gooch crucible containing glass wool, and the filtrated was stored at 5°C until needed. Freezing point depressions were run on the hemolymph and fatty acid-albumin complex, along with various standards, in order to correct the complex so it would be isotonic to the hemolymph.

Preparation of Tissue: Adult female Ascaris lumbricoides suum were obtained from Wilson and Company, Oklahoma City, Oklahoma. The worms were transported to the laboratory in saline solution (14) which was maintained at 32-40°C.

Worms were sliced longitudinally. The gut was removed and immediately placed in a large petri dish containing saline at 37°C. The midgut was used in all determinations and was inverted by the following procedure. A pasture pipette was inserted the length of the gut and the

end of the gut near the tip of the pipette was tied securely to the pipette. The pipette was then withdrawn from the gut and in so doing, the gut was turned with the mucosal side out. The inverted gut was flushed first with warm saline and then with hemolymph by passing the fluids through the pipette. The free end of the gut was then tied, with thread, and the sac preparation was filled with hemolymph.

Incubation: The pipette and attached gut were placed in a test tube containing 5 ml of the fatty acid-albumin complex and incubated for 1 hour at 37°C. At the end of this period, the hemolymph, gut, and a 1 ml aliquot of the fatty acid-albumin complex were extracted separately with chloroform-methanol (2:1) overnight. Following extraction, the chloroform solution was filtered in the cold and dried over $NaSO_4$, for 1 hour. The $NaSO_4$ was removed by filtration and the chloroform was removed in vacuo at 40°C. Residue lipid was redissolved in 0.5 ml of chloroform.

Counting: 0.1 ml of the chloroform solution was placed in a scintillation vial with 10 ml of scintillation fluid and counted with a Packard Tricarb Liquid Scintillation Spectrometer. The remaining lipids of each sample were separated into their various classes by thin layer chromatography. Separation was carried out by running each chromatogram in hexane-ether-acetic acid (60:40:1). The spots were visualized by placing the plate in a jar containing iodine crystals. Each spot was eluted from the silica gel with chloroform, concentrated to a known volume and counted in a liquid scintillation spectrometer.

Identification of the Various Spots: Iodine vapor, surfuric acid and charring, and Antimony Trichloride were used as general detecting sprays for the lipid spots in question (20). Specific sprays were

employed to further characterized individual spots. The phospholipids were detected by using Ammonium Molybdate-Perchloric Acid spray (22). Antimony Trichloride was employed to detect cholesterol, as well as other sterols and sterol esters (20). Free fatty acids were visualized by Bromocresol Green (18). Hydroxylamine Ferric Chloride which detects esters was used to identify the glyceride spots. A series of standard triglycerides which included tripalmitin were employed for further identification.

Thin layer chromatography did not provide adequate separation of some components which were hard to identify. One spot was eluted from the thin layer plate with chloroform. The eluted lipids was refluxed for 2 hours with 10% methanolic KOH and a small amount of water. The unsaponifiable fraction was separated by washing the basic solution with pentane. The pentane was then washed with water to insure removal of soaps. The resultant unsaponifiable fraction was run again on a thin layer plate and the spots were visualized by spraying with Antimomy Trichloride.

CHAPTER III

RESULTS

Movement of Radioactivity: The transport of palmitic acid-1-C^{14} across the gut of Ascaris was measured under four different experimental conditions. These included aerobic and anaerobic environments and the presence and absence of nutrients and iodoacetic acid. Five determinations were carried out for each of the four experiments. Total activity after incubation (based on initial activity) was determined for the intestinal wall, and the mucosal and serosal fluids. The results from these experiments are presented in Table I. After incubation under anaerobic conditions for 1 hour, it was found that approximately 87.0 percent of the original activity remained on the mucosal side of the gut. The intestinal wall contained an average of 6.9 percent of the original activity and the serosal compartment contained approximately 6.0 percent of the activity. When glucose was omitted from the anaerobic system, it was found that approximately 92.3 percent of the original activity remained on the mucosal side of the gut. The intestinal wall and serosal compartment had 4.3 percent and 3.4 percent of the original activity, respectively. The least significant difference test (lsd) was used in evaluating the significance between means of the various experiments. The lsd value (the minimum difference that two means could differ and be significant) for the mucosal fluid in the complete system and the system minus glucose was calculated to be 4.88 percent at the 0.05 probability

level. The observed difference between the two means is 5.3 percent.

Therefore, the means differ significantly in the presence and absence of glucose. The calculated lsd value for the intestine was 2.8 percent at the 0.05 probability level while the experimental data give a value of 2.6 percent. Thus statistically there was no significant difference between the two. The calculated lsd value for the serosal fluid was 2.59 percent at the 0.05 probability level while the observed value was 2.60 percent which is slightly above the value determined statistically. The results suggest that palmitic acid moves across the gut and that glucose stimulates this movement.

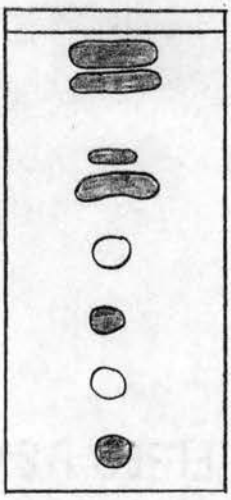
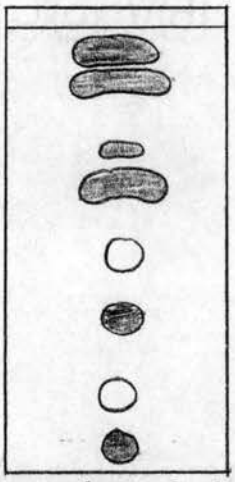
In determinations with iodoacetic acid the mucosal side of the gut contained 89.9 percent of the original radioactivity. The intestinal wall contained 5.8 percent of the activity and the serosal compartment contained 4.3 percent. The lsd value for the mucosal fluid in the complete system and the system containing iodoacetic acid was calculated to be 4.88 percent at the 0.05 probability level. The observed difference between the two means is 2.8 percent. Hence, the means do not differ significantly in the presence and absence of iodoacetic acid. The calculated lsd value for the intestine was 2.8 percent at the 0.05 probability level while the experimental data gave a value of 1.1 percent. Thus, statistically there was no significant difference between the two. The calculated lsd value for the serosal fluid was 2.59 percent at the 0.05 probability level while the experimental value was 1.7 percent which is below the value determined statistically. Although the difference between the complete system and the system containing iodoacetic acid were not significant at the 0.05 probability level, inspection of the results suggest that there is some reduction in the movement of radioactivity.

TABLE I

TOTAL ACTIVITY RECOVERED FROM MUCOSAL SOLUTION INTESTINAL WALL AND SEROSAL SOLUTION AFTER C^{14}-PALMITIC ACID-ALBUMIN COMPLEX WAS PLACED ON MUCOSAL SIDE OF THE ISOLATED INTESTINE.

	Total Activity After Incubation Based on Initial Activity		
System	Mucosal %	Intestinal Wall %	Serosal %
Anaerobic (95% N_2 + 5% CO_2)			
Complete*	85.2	9.1	5.7
"	85.0	8.9	6.1
"	87.0	4.7	8.2
"	85.9	7.3	6.7
"	91.9	4.7	3.2
Average	87.0	6.9	6.0
Minus Glucose	93.0	3.6	3.3
"	93.0	4.7	2.6
"	89.0	5.9	4.9
"	93.0	3.8	3.1
"	93.5	3.3	3.0
Average	92.3	4.3	3.4
Complete + IAA	87.6	8.2	4.0
"	92.5	4.6	2.8
"	91.8	2.5	5.6
"	87.9	5.2	6.8
"	89.0	8.4	2.4
Average	89.8	5.8	4.3
Aerobic (air)			
Plus Glucose	88.1	5.0	7.0
"	94.9	2.9	2.0
"	86.9	7.5	7.5
"	92.6	3.2	4.0
"	91.1	5.7	3.6
Average	90.7	4.9	4.8

*Complete System-0.043 M Glucose plus Anaerobic Atmosphere (95% N_2 5% CO_2).

Spot No.	Chromatograms	$\%C^{14}$	Tentative Identification
	HEMOLYMPH		
8		0.3 (4)*	Sterol Ester
7		1.6 (4)	Triglyceride
6		0.2 (4)	Ascaroside Ester
5		91.8 (4)	Free Fatty Acid
4		-	Unknown
3		5.6 (4)	Ascaroside Ester ?
		-	Cholesterol
2		-	Unknown
1		0.6 (4)	Phospholipids
	GUT		
8		0.7 (4)	Sterol Ester
7		22.5 (4)	Triglyceride
6		1.4 (4)	Ascaroside Ester
5		66.7 (4)	Free Fatty Acid
4		-	Unknown
3		7.4 (4)	Ascaroside Ester ?
		-	Cholesterol
2		-	Unknown
1		1.5 (4)	Phospholipids

*(4) Indicates number of observation
Solvent system: (hexane: ether: acetic acid) (60:40:1)

Figure 4. Analysis of Lipids.

In the experiments where the gut was incubated with glucose, the mucosal side of the gut contained 90.7 percent of the original activity. The intestinal wall had 4.9 percent of the activity and the serosal compartment 4.8 percent. The lsd value for the mucosal fluid in the complete system and the system utilizing an aerobic atmosphere was calculated to be 4.88 percent at the 0.05 probability level. The observed difference between the two means is 3.7 percent. Therefore, the means do not differ significantly in the presence and absence of an aerobic atmosphere. The calculated lsd value for the intestine was 2.8 percent at the 0.05 probability level while the experimental data gave a value of 2.0 percent. Thus, statistically there was no significant difference between the two. The calculated lsd value for the serosal fluid was 2.59 percent at the 0.05 probability level while the observed value was 1.2 percent which is below the value determined statistically. When compared to the complete system, there seems to be less transport across the gut wall under aerobic condition, even though, the results were not statistically significant at the 0.05 probability level.

Analysis of Lipids: In all experiments the lipids were separated into their various classes by thin layer chromatography and the distribution of radioactivity was determined. The results of these analysis are presented in Figure 5.

The Rf. values were determined and each spot was given a number. The Rf. values of various standards were determined and compared with those of the spots in question.

Spots 2 and 4 were not always present in each of the runs. Thus, identification was not possible and they are labeled unknown.

Spot number 1 gave a dark brown positive reaction when sprayed with

Ammonium Molybdate-Perchloric Acid, a reagent that is frequently used to identify phospholipids. Further, phospholipid standards remain at the origin of chromatograms eluted with hexane:ether:acetic acid (60:40:1). For these reasons, spot number 1, in both the hemolymph and gut tissue, was characterized as phospholipids. These lipids contained less than 2.0 percent of the total radioactivity incorporated by either tissue.

Spot number 3 gave a pink color when sprayed with Antimony Trichloride and the color became purple after the thin layer plate was heated in the oven for 5 minutes at 105°C. This color response is characteristic for cholesterol and the Rf. value for the spot agrees very well with values obtained with a cholesterol standard. In addition to the positive test with Antimony Trichloride this spot also gave a positive test for a sugar when p-Dimethylaminobenzaldehyde was the spray reagent. Ascarosides and their esters contain one or two hexose sugars in their molecular structure. It was assumed that the spot was a mixture of cholesterol and an Ascaroside or its ester. In an effort to determine if the spot was more than just cholesterol, it was eluted from the plate and run twice in benzene:ethyl acetate (2:1) and once in hexane: ether:acetic acid (60:40:1). When the rechromatographed lipid was sprayed with Antimony Trichloride, it was found that three spots were present, as shown in Figure 5, but only one spot gave a positive test for cholesterol.

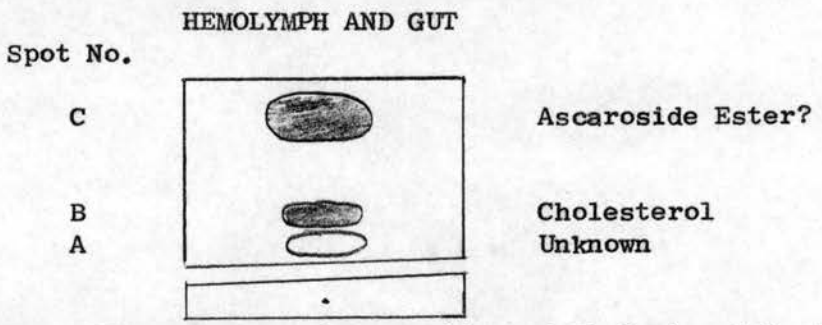


Figure 5. Thin Layer Chromatogram of Separation of Cholesterol Spot.

Spot C is quite probably an Ascaroside ester, however, no positive test with p-Dimethylaminobenzaldehyde was obtained upon spraying the rechromatographed lipid. Since only a small quantity of the eluted lipid was available for analysis, it is probable that this was less than the minimum amount required for a positive test with this reagent. Each spot was eluted and counted with the Packard Tricarb Spectrometer. The Ascaroside ester (Spot C) was found to contain the entire radioactivity. Cholesterol in the spot did not contain radioactivity.

Spot 5 gave an immediate positive test for acid with Bromocresol Green reagent and the Rf. value for the spot corresponded with standard non-volatile fatty acids. For further identification, spot 5 was eluted and rechromatographed. A solution of palmitic acid standard was run beside the eluted spot, and the chromatogram was sprayed with Bromocresol Green. Both the palmitic acid standard and free fatty acid spot gave a positive yellow color on a green background. The free fatty acid contains the greatest amount of activity, approximately 92.0 percent in the hemolymph and 67.0 percent in the gut tissue.

Spot 6 did not give a positive test for a sugar when p-Dimethylaminobenzaldehyde was the spray reagent. Since only a small quantity of the eluted lipid was available for analysis, it is probable that this was less than the minimum amount required for a positive test with this reagent. However, when the lipids from 63 ml. of hemolymph were obtained and analysed by thin layer chromatography the spot in question gave a positive test for sugar. Therefore, spot 6 is an Ascaroside ester. This Ascaroside ester was found to contain less than 2.0 percent of the total radioactivity in both the hemolymph and gut tissue.

Spot 7 gave a positive color reaction with Hydroxylamine Ferric Chloride and the Rf. value for the spot corresponded with standard triglycerides. For further identifications, spot 7 was eluted and re-chromatographed. Standard solution of various triglycerides was run along side the eluted spot, and the chromatogram was sprayed with Hydroxylamine Ferric Chloride. Both the triglyceride standards and spot 7 gave a positive purple color on a white background. In the triglyceride spot, there was a great difference in the amount of activity in the hemolymph and gut tissue. The triglyceride spot of the hemolymph contained only about 1.6 percent of the total activity; whereas, the same spot in the gut tissue contained approximately 22.5 percent of the activity.

Spot 8 gave a positive color reaction for a sterol with Antimony Trichloride, and the Rf. value corresponds with cholesterol acetate. The spot was eluded from the thin layer plate with chloroform and saponified with methanolic potassium hydroxide. The methanol was evaporated to about a half of its original volume and diluted with an equal volume of water. The aqueous solution was extracted with ether and the ether was separated and removed in vacuo at 40°C. The lipid residue was taken up in chloroform and spotted on a thin layer plate. The plate was run in hexane:ether:acetic acid (60:40:1) and sprayed with Antimony Trichloride reagent. After the plate had been heated for about 15 minutes at 105°C, the characteristic purple color had an Rf. value corresponding to cholesterol. This strongly suggest that spot 8 is a cholesterol ester.

Distribution of Radioactivity: Radioactivity was concentrated in the Ascaroside ester, free fatty acid, and triglycerides of both the hemolymph and gut. The free fatty acid spot of the hemolymph contained nearly 92.0 percent of the entire radioactivity. In the gut tissue, the free

fatty acid spot and the triglyceride spot also contained approximately 90.0 percent of the total radioactivity. The phospholipids and sterol ester of both the hemolymph and gut tissue contained radioactivity that was less than 2.0 percent of the entire radioactivity.

Table II summarizes the entire set of results obtained from the two sets of experiments in which the radioactivity of each spot was determined and the identity of the spots were resolved.

When comparing the percent of radioactivity obtained for the free fatty acid from the various systems utilizing an anaerobic atmosphere, little difference is noted between them in the hemolymph or gut. However, when these results are compared with those of the aerobic atmosphere, there is a definite difference. In the anaerobic atmosphere the radioactivity is concentrated primarily in the free fatty acids; whereas, in the system utilizing an aerobic atmosphere the radioactivity is present in the free fatty acid, but there is also an increase in the radioactivity present in the triglycerides and Ascaroside esters.

TABLE II

DISTRIBUTION OF RADIOACTIVITY IN LIPIDS OF
ASCARIS TISSUE FOLLOWING INCUBATION
IN PALMITIC ACID-1-C^{14}

Lipids	Percent of the Total Radioactivity							
	Hemolymph				Gut			
	Aerobic	Anaerobic			Aerobic	Anaerobic		
	Complete	Complete	Plus IAA	Minus Glucose	Complete	Complete	Plus IAA	Minus Glucose
Phospholipids	0.7	0.5	0.5	0.5	1.7	1.0	1.7	1.7
Triglyceride	2.4	1.8	0.9	1.4	24.7	21.4	19.7	24.2
Non-volatile Fatty Acids	89.0	94.0	91.0	93.0	58.7	71.1	68.2	68.9
Sterol esters	0.2	0.4	0.2	0.2	0.5	0.8	1.0	0.5
Cholesterol	-	-	-	-	-	-	-	-
Ascaroside esters	7.8	3.4	7.4	4.9	14.4	5.7	9.5	5.5

Each of the values is an average of 4 runs.
Results from TLC (solvent: hexane:ether:acetic acid (60:40:1)).
Aerobic-air: Anaerobic 95% N_2-5% CO_2.

CHAPTER IV

DISCUSSION

The four experiments differed in either the atmosphere in which the runs were made or the consitituents present in the medium. In all of the experiments radioactivity moved from the mucosal side to the serosal side of the gut. The greatest movement was observed under anaerobic conditions (95% N_2-5% CO_2) with glucose added to the mucosal fluid.

When glucose was omitted from the medium less radioactivity was moved across the intestinal wall, and this reduction in movement was statistically significant at the 0.05 probability level. Thus, the presence of glucose does enhance the movement of radioactivity. This suggests that metabolic energy is involved in the movement of radioactivity.

It is well known that carbohydrate metabolism in Ascaris occurs via the Embden-Meyerhof pathway (2,21). Although, some nematodes possess all of the components of the tricarboxylic acid cycle, Ascaris contains only part of the cycle (2, 5, 19, 21). Thus, it is probable that the bulk of the metabolic energy comes from the reactions occurring in glycolysis.

With this in mind, an effort was made to inhibit the Embden-Meyerhof pathway in the cells of Ascaris gut by introducing the inhibitor iodoacetic acid. Iodoacetic acid reacts with among other things, sulfhydryl groups of the glycolytic enzyme phosphoglyceraldehyde dehydrogenase (triose phosphate dehydrogenase), and inactiviates the enzyme (23). In the presence of iodoacetic acid, a reduction in the movement of radioactivity was ob-

served. However, when the results were compared to the complete system, it was seen that there was no significant difference between the two systems at the 0.05 probability level. This slight reduction in transport is possibly due to the inhibitor blocking glycolysis, thus, causing a reduced energy source.

Although the more "normal" gas phase for the cells of Ascaris gut is one low in or lacking oxygen, a series of experiments were carried out with air passing throughthe incubation medium. The rate of movement of radioactivity across the gut was reduced by the presence of oxygen. Statistically however, there was no significant difference at the 0.05 probability level between the anaerobic and aerobic atmosphere. Never the less, the results were constant and it seems possible that an anaerobic atmosphere enhances movement of radioactivity across the gut of Ascaris. This is in contrast with results reported by Johnston (15) on transport of C^{14}-palmitic acid in the small intestine of the golden hamster. He found that when an aerobic atmosphere was utilized, the transport was greatly enhanced over the system using an aerobic atmosphere. It was observed that 5.0 percent of the original activity that had been place on the mucosal side was present in the serosal fluid when 95% O_2-5% CO_2 was employed as the gas phase but only 0.25 percent of the activity was contained in the serosal fluid when 95% N_2-5% CO_2 was bubbled through the system. Thus, in the mammalian organism an aerobic atmosphere enhances the transport of palmitic acid across the intestinal wall.

The results with Ascaris agree with Harpur (9) who found that air has a deleterious effect upon the metabolism of the tissues of the worm and the effect becomes more pronounced with time. Thus, he recommends

that nitrogen be used as the gas phase, in order to create an anerobic environment.

When all four experiments were compared, it was found that an energy source and an anaerobic atmosphere are factors that have a definite effect on the movement of radioactivity across the gut wall. The fact that a movement of radioactivity does occur, is stimulated by glucose, and perhaps inhibited by iodoacetic acid certainly suggests an active transport mechanism in the worm.

When the distribution of radioactivity in the lipids of the Ascaris hemolymph and gut tissue was determined, over 50 percent of the radioactivity was found in the free fatty acids in the hemolymph and gut tissue. A significant amount of the activity was in the triglycerides. Thus, in the gut tissue the fatty acids may go through a series of steps in which they are transformed into triglycerides much as occurs in mammalian tissue. However, there was no evidence found indicating that monoglycerides and diglycerides were present in either the hemolymph or gut tissue. When comparing the hemolymph and gut tissue it seemed that triglycerides of the gut tissue must be hydrolyzed and the free fatty acids released into the hemolymph due to the high percent of activity present in the fatty acid of the hemolymph.

The phospholipids were found to contain very little of the total activity in both the hemolymph and gut.

When referring to Table II, it can be seen that an aerobic atmosphere reduces the precentage of free fatty acids and increases the concentration of glyceride and Ascaroside ester. No immediate explanation is available for these results.

The results of this study suggest that Ascaris is capable of acquiring non-volitile fatty acid by a process of active transport. Apparently, this process functions best under anaerobic conditions, a situation which is the opposite of fatty acid transport in the vertebrate intestine. Evolutionary processes have apparently selected a transport mechanism for Ascaris which best adapts it for survival in its normal anaerobic environment.

CHAPTER V

SUMMARY AND CONCLUSIONS

Experiments have been carried out to determine the manner in which palmitic acid is transported across the intestinal wall of Ascaris. Lipids of the hemolymph and gut tissue were analyzed and the distribution of the radioactivity was determined. The following results were obtained.

1. When an energy source was added to the system, the movement of palmitic acid across the intestinal wall was enhanced. The inhibitor, iodacetic acid, when added to the complete system caused a reduction in the movement of palmitic acid across the intestinal wall, however, the reduction was not significant at the 0.05 probability level.

2. When an anaerobic atmosphere was employed and the results of the experiments were compared with those of a system utilizing an aerobic atmosphere, it was found that the movement was enhanced when the anaerobic atmosphere was used. The difference in the two systems was not significant however at the 0.05 probability level.

3. The distribution of radioactivity in the lipids of Ascaris gut tissue and hemolymph was determined. Approximately 66.0 percent of the radioactivity in the intestinal wall was present as the free fatty acid. Approximately 22.0 percent of the radioactivity in the gut was incorporated into triglycerides.

In the hemolymph (the fluid on the serosal side of the gut) approximately 80.0 percent of the radioactivity was present as the free fatty acid, and 1.6 percent was found in the triglycerides.

This study opens up a new field of endeavor. There are many experiments that can be done in order to further the study of lipid transport. At the present, further studies are being run in order to determine the mechanisms involved in the transport of both palmitic acid and glucose. Other experiments involving the transport of labeled substances such as monoglycerides, diglycerides, or triglycerides are also possibilities.

BIBLIOGRAPHY

(1) Borgström, Bengt. "Absorption of Triglycerides." In Proc. Intern. Symp. on Lipid Transport. Edited by H. C. Meng. Springfield, Illinois. (1964) 15-21.

(2) Bueding, Ernest. "Comparative Aspect of Carbohydrate Metabolism." Fed. Proc. Vol. 21 (1962) 1039-1046.

(3) Carpenter, M. F. "The digestive enzymes of Ascaris lumbricoides, var. suum: Their Properties and Distribution in the Alimentary Canal." Ph.D. Dissertation, University of Michigan, Detroit, Michigan. (1952)

(4) Chitwood, B. G. and M. B. Chitwood. "An Introduction to Nematology: Section I. Anatomy." Monumental Printing Company, Baltimore, Maryland. (1959) 23-32.

(5) Entner, N. "The Occurrence of the Pentose Phosphate Pathway in Ascaris lumbricoides." Arch. Biochem. Biophys. Vol. 71. (1957) 52-61.

(6) Feist, C. F., C. P. Read, and F. M. Fisher, Jr. "Trehalose Synthesis and Hydrolysis in Ascaris suum. J. of Parasital. Vol. 51. (1962) 76-78.

(7) Fisher, F. M., Jr. "Methods for the Study of Transport Mechanisms in the Isolated Intestine of Ascaris lumbricoides." J. of Parasitol. Vol. 48. (1962) 26-27.

(8) Fredrickson, R. S., and R. S. Gordon, Jr. "Transport of Fatty Acids." Physiol. Rev. Vol. 38. (1958) 585-630.

(9) Garven, H. S. D. "The Digestive System." In A Student's Histology. E. & S. Livingstone LTD., Edinburgh. (1957) 333-425.

(10) Guyton, Arthur, C. "Lipid Metabolism." In Textbook of Medical Physiology. W. B. Saunders Company, Philadelphia. (1962) 889-903.

(11) Harpur, R. P. "Maintenance of Ascaris lumbricoides in Vitro. A Biochemical and Statistical Approach." Can. J. Zool. Vol. 40. (1962) 991-1011.

(12) Hoffmann, A., and B. Borgström. "Physico-chemical State of Lipids in Intestinal Content During Their Digestion and Absorption." Fed. Proc. Vol. 21. (1962) 42-50.

(13) Isselbacker, Kurt J. "Metabolism and Transport of Lipid by Intestinal Mucosa." Fed. Proc. Vol. 24. (1965) 16-22.

(14) Jacobson, N. S. "Lipid Metabolism in Female Ascaris lumbricoides During Starvation." Ph. D. Dissertation, Oklahoma State University, Stillwater, Oklahoma. (1965).

(15) Johnston, John M. "An In Vitro Study of Fatty Acid Absorption." P. S. E. B. M. Vol. 98. (1958) 836-838.

(16) Johnston, J. M., and J. H. Bearden. "Intestinal Phosphatidate Phosphatase." Biochem. Biophys. Acta. Vol. 56. (1962) 365-367.

(17) Kessel, R. G., J. J. Prstage, S. S. Seknon, R. L. Smalley, and H. W. Beams. "Cytological Studies on the Intestinal Epithelial Cells of Ascaris lumbricoides suum." Trans. of the Amer. Micro. Soc. Vol. 80. (1961) 103-118.

(18) Lange, Norbert Adolph. Handbook of Chemistry. Handbook Publisher, Inc., Sandusky, Ohio. (1944) 1120.

(19) Lee, D. L. "Feeding and Digestion." In The Physiology of Nematodes. W. H. Freeman and Company, San Francisco. (1965) 15-37.

(20) Mangold, Helmut K. "Thin-Layer Chromatography of Lipids." J. Amer. Oil Chem. Soc. Vol. 38. (1961) 708-727.

(21) Read, C. P. "The Carbohydrate of Worms." In Comparative Physiology of Carbohydrate Metabolism in Heterothermic Animals. Edited by A. W. Martin, University of Washington, Seattle, Washington. (1961) 3-34.

(22) Wagner, Hildebert, Ludwig Horhammer, and Petter Wolff. "Dunnschichtchromatographie von phosphatiden und glykolipiden." Biochem. Z. Vol. 334. (1961) 175-184.

(23) White, A., Philip Handler, and Emil L. Smith. "Carbohydrate Metabolism." In Principles Biochemistry. McGraw-Hill Book Company, New York. (1964) 362-400.

(24) White, A., Philip Handler, and Emil L. Smith. "Lipid Metabolism." In Principles Biochemistry. McGraw-Hill Book Company, New York. (1964) 429-462.

Appendix

ANALYSIS OF VARIANCE

MUCOSAL SIDE

N = 20
t = 4
r = 5

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square
Total	N-1=	270.85	
Between Treatments	t-1= 3	74.79	24.93
Within Treatments	N-t= 16	196.06	12.25

System 1	System 2	System 3	System 4
$\xi x_i = 435.0$	$\xi x_i = 461.5$	$\xi x_i = 447.8$	$\xi x_i = 453.6$
$\xi x_i^2 = 37,877.46$	$\xi x_i^2 = 42,610.25$	$\xi x_i^2 = 40,212.16$	$\xi x_i^2 = 41,193.20$

$$\text{Total SS} = x_{ij}^2 - \frac{(\xi x_{ij})^2}{N} = 161,893.07 - \frac{(1797.9)^2}{20} = 161893.07 - 161622.22 = 270.85$$

$$\text{TSS} = \frac{(\xi x_i)^2}{r} - \frac{(\xi x_{ij})^2}{N} = \frac{(435.0)^2 + (461.5)^2 + (447.8)^2 + (453.6)^2}{5} - \frac{(1797.9)^2}{20} =$$

$161697.01 - 161622.22 = 74.79$

ESS=difference = 270.85-74.79 = 196.06

$$\text{TMS} = \frac{\text{TSS}}{t-1} = \frac{74.79}{3} = 24.93$$

$$\text{EMS} = \frac{\text{ESS}}{N-t} = \frac{196.06}{16} = 12.25$$

$$lsd_{.05} = t_{.05}(16) \quad MS(2/5)$$

$$lsd_{.05} = 2.120 \cdot 12.25(2/5)$$

$$2.120 \cdot 2.3 = 4.88$$

$$lsd_{0.1} = 1.746 \cdot 2.3 = 4.02$$

ANALYSIS OF VARIANCE

INTESTINAL WALL

N = 20
t = 4
r = 5

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square
Total	N-1= 19	91.06	
Between Treatments	t-1= 3	21.82	7.27
Within Treatments	N-t= 16	69.24	4.33

System 1	System 2	System 3	System 4
$\xi x_i = 34.7$	$\xi x_i = 21.3$	$\xi x_i = 28.9$	$\xi x_i = 23.3$
$\xi x_i^2 = 259.49$	$\xi x_i^2 = 95.19$	$\xi x_i^2 = 192.25$	$\xi x_i^2 = 129.49$

$$\text{Total SS} = \xi x_{ij}^2 - \frac{(\xi x_{ij})^2}{N} = 676.42 - \frac{(108.2)^2}{20} = 676.42 - 585.36 = 91.06$$

$$\text{TSS} = \frac{(\xi x_i)^2}{r} - \frac{(\xi x_{ij})^2}{N} = \frac{(34.7)^2 + (21.3)^2 + (28.9)^2 + (23.3)^2}{5} - \frac{(108.2)^2}{20} = 607.18 - 585.36 = 21.82$$

ESS = difference = 91.06 - 21.82 = 69.24

$$\text{TMS} = \frac{\text{TSS}}{t-1} = \frac{21.82}{3} = 7.27$$

$$\text{EMS} = \frac{\text{ESS}}{N-t} = \frac{69.24}{16} = 4.33$$

$$lsd_{.05} = t_{.05}(16) \quad MS(2/5)$$

$$lsd_{.05} = 2.120 \cdot 4.33\,(2/5)$$
$$2.120 \cdot 1.32 = 2.80$$

$$lsd_{0.1} = 1.746 \cdot 1.32 = 2.30$$

ANALYSIS OF VARIANCE

SEROSAL COMPARTMENT

N = 20
t = 4
r = 5

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square
Total	N-1= 19	77.14	
Between Treatments	t-1= 3	17.35	5.78
Within Treatments	N-t= 16	59.79	3.74

System 1	System 2	System 3	System 4
$\xi x_i = 29.9$	$\xi x_i = 16.9$	$\xi x_i = 21.6$	$\xi x_i = 23.1$
$\xi x_i^2 = 192.07$	$\xi x_i^2 = 60.27$	$\xi x_i^2 = 107.20$	$\xi x_i^2 = 136.21$

$$\text{Total SS} = \xi x_{ij}^2 - \frac{(\xi x_{ij})^2}{N} = 495.75 - \frac{(91.5)^2}{20} = 495.75 - 418.61 = 77.14$$

$$\text{TSS} = \frac{(\xi x_i)^2}{r} - \frac{(\xi x_{ij})^2}{N} = \frac{(29.9)^2+(16.9)^2+(21.6)^2+(23.1)^2}{5} - \frac{(91.5)^2}{20} = 435.96 - 418.61 = 17.35$$

ESS = difference = 77.14 - 17.35 = 59.79

$$\text{TMS} = \frac{\text{TSS}}{t-1} = \frac{17.35}{3} = 5.78$$

$$\text{EMS} = \frac{\text{ESS}}{N-t} = \frac{59.79}{16} = 3.74$$

$$lsd_{.05} = t_{.05}(16) \quad MS(2/5)$$

$$lsd_{.05} = 2.120 \cdot 3.74(2/5)$$
$$2.120 \cdot 1.22 = 2.59$$

$$lsd_{0.1} = 1.746 \cdot 1.22 = 2.13$$

COMPARISON

	Mucosal	Intestine	Serosal
	lsd=4.88	lsd=2.80	lsd=2.59
(Minus glucose)-Complete	92.3-87.0=5.3	6.9-4.3=2.6	6.0-3.4=2.6
(IAA) - Complete	89.8-87.0=2.8	6.9-5.8=1.1	6.0-4.3=1.7
(Aerobic) - Complete	90.7-87.0=3.7	6.9-4.9=2.0	6.0-4.8=1.2

VITA

Gary Allen King

Candidate for the degree of

Master of Science

Thesis: TRANSPORT OF PALMITIC ACID ACROSS THE GUT OF ASCARIS LUMBRICOIDES SUUM

Major Field: Physiology

Biographical:

Personal Data: Born November 5, 1941, in Stillwater, Oklahoma, the son of Merle A. and Helen L. King.

Education: Attended elementary and secondary school at Stillwater, Oklahoma; graduated from Stillwater High School in 1960; received the Bachelor of Science degree from Oklahoma State University, Stillwater, Oklahoma in May, 1964, with a major in physiology. Completed requirements for the Master of Science degree at Oklahoma State University, Stillwater, Oklahoma in July, 1966.

Professional Experience: Served as a Comparative Physiology and Cellular Physiology laboratory assistant for 1 year at Oklahoma State University; laboratory technician in a physiology laboratory, Department of Physiology, Oklahoma State University; served as teaching assistant in the Department of Physiology and Pharmacology for the Spring semester of 1966, Oklahoma State University.